

IN THE CLAIMS:

1-171. (previously cancelled).

172-189. (cancelled).

190. (currently amended) ~~The method of claim 172, wherein steps (b) and (c) comprise the sub-steps of:~~ A method for making a transcription product having a sequence corresponding to a target sequence in a target nucleic acid; the method comprising the steps of:

(a+) primer extending a sense promoter primer that exhibits a sense promoter sequence in its 5'-end portion and a sequence complementary to target sequence at its 3'-end with a DNA polymerase using the target nucleic acid in the sample as a template to generate obtain the single-stranded DNA comprising the target sequence, which single-stranded DNA comprises linear sense promoter-containing first-strand cDNA; and

(b) removing the target nucleic acid;

(cb+) circularizing the linear sense promoter-containing first-strand cDNA with a ligase, to thereby operably joining the single-stranded DNA comprising the target sequence to the sense promoter sequence in its 5'-end portion to the target-complementary sequence at its 3'-end;

(d) to obtaining a circular single-stranded transcription substrate;

(e) admixing the circular single-stranded transcription substrate with an RNA polymerase and NTPs and incubating under conditions wherein a transcription product is synthesized.

191. (currently amended) The method of claim 190, ~~the method~~ additionally comprising the step of cleaving the circular ~~single-stranded~~ transcription substrate at a site that is 3'-of the promoter sequence and 5'-of the target-complementary sequence to generate obtain a linear ~~single-stranded~~ transcription substrate.

192. (previously presented) The method of claim 190, wherein the target nucleic acid in the sample comprises RNA such as mRNA, or a transcription product, and the DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity.

193-197. (cancelled)

198-205. (cancelled)

206. (new) The method of claim 190, wherein the sense promoter primer is selected from the group consisting of:

- (i) an oligo(dT) promoter primer;
- (ii) an anchored oligo(dT) promoter primer;
- (iii) a specific-sequence promoter primer that is complementary to a specific sequence in the target nucleic acid sequence; and
- (iv) a random sequence promoter primer that exhibits a random sequence at its 3'-end.

207. (new) The method of claim 190, wherein the sense promoter primer additionally comprises one or more transcription termination sequences between the target-complementary sequence at its 3'-end and the sense promoter sequence in its 5'-end portion.

208. (new) The method of claim 190, wherein the sense promoter primer additionally comprises a transcription initiation sequence 5'-of the sense promoter sequence.

209. (new) The method of claim 190, wherein, between the target-complementary sequence at its 3'-end and the sense promoter sequence in its 5'-end portion, the sense promoter primer additionally comprises one or more sequences or genetic elements selected from among one or more origins of replication, one or more sequences that encode a selectable or screenable marker, one or more sequences that can be recognized and used by a transposase for in vitro or in vivo transposition, and one or more sites that are recognized by a recombinase.

210. (new) The method of claim 191, wherein the sense promoter primer has a dUMP nucleotide between the target-complementary sequence at its 3'-end and the sense promoter sequence in its 5'-end portion.

211. (new) The method of claim 210, wherein the circular transcription substrate is linearized by treatment with uracyl-N-glycosylase (UNG) and endonuclease IV (endo IV).

212. (new) The method of claim 190, wherein the ligase used in step (c) for said joining is a thermostable RNA ligase derived from phage TS2126 which infects *Thermus scotoductus*.

213. (new) The method of claim 190, wherein the method is performed in a stepwise fashion by purifying the reaction products by removing reaction components and/or inactivating enzymes from one set of reactions prior to proceeding to the next set of reactions.

214. (new) The method of claim 213, wherein the linear sense promoter-containing first-strand cDNA reaction product is purified prior to the step of circularizing with a ligase.

215. (new) A method for generating linear first-strand cDNA complementary to a target sequence in a target nucleic acid wherein its 3' and 5' ends exhibit sequences that are not complementary to the target sequence, the method comprising:

- (1) primer extending a primer with a DNA polymerase using target nucleic acid as a template to generate linear first-strand cDNA that is complementary to the target sequence, wherein the primer comprises (i) a 5'-end portion that is not complementary to the target nucleic acid, (ii) a 3'-end portion that is complementary to the target nucleic acid, and (iii) a cleavage site within its 5'-end portion;
- (2) purifying the linear first-strand cDNA reaction products by removing reaction components and/or inactivating enzymes from the DNA polymerase primer extension reaction of step (1);
- (3) circularizing the linear first-strand cDNA generated in step (1) with a ligase under ligation conditions to generate circular first-strand cDNA;
- (4) purifying the circular first-strand cDNA by removing reaction components and/or inactivating enzymes from the ligase reaction of step (3);
- (5) linearizing the circular first-strand cDNA generated in step (3) at the cleavage site to generate linear first-strand cDNA that exhibits the sequence 3'-of the cleavage site of the primer in its 5'-end portion and the sequence 5'-of the cleavage site of the primer in its 3'-end portion;

and

(6) using the linear first-strand cDNA generated in step (5) for sequencing of the target nucleic acid.

216. (new) The method of claim 215, wherein the ligase for circularizing is the RNA ligase derived from phage TS2126 that infects *Thermus scotoductus*.

217. (new) The method of claim 215, wherein the cleavage site in the primer comprises a dUMP nucleotide.

218. (new) The method of claim 217, wherein said step of linearizing the circular first-strand cDNA comprises treating with uracyl-N-glycosylase (UNG) and endonuclease IV.

219. (new) The method of claim 215, wherein the target nucleic acid is RNA and the primer is selected from the group consisting of:

- (i) an oligo(dT) primer;
- (ii) an anchored oligo(dT) primer;
- (iii) a specific-sequence primer that is complementary to a specific sequence in the target nucleic acid sequence; and
- (iv) a random sequence primer that exhibits a random sequence at its 3'-end.